The Periodate Oxidation of Amino Acids with Reference to Studies on Glycoproteins

BY J. R. CLAMP* AND L. HOUGH
Department of Organic Chemistry, University of Bristol

(*Received* 26 *March* 1964)

1. All α-amino acids are oxidized by periodate, but at different rates. 2. The rates of oxidation of individual α-amino acids vary with pH. In general, oxidation proceeds more rapidly at alkaline pH. 3. Serine, threonine, cysteine, cystine, methionine, proline, hydroxyproline, tryptophan, tyrosine and histidine are rapidly and extensively oxidized by periodate. 4. Cysteine, cystine, methionine, tryptophan, tyrosine and histidine are oxidized by periodate when they are substituted in the carboxyl and amino groups, as in a polypeptide chain.

To evaluate the use of the periodate oxidation method for the structural investigation of carbohydrates found in combination with proteins, peptides and amino acids, as in glycoproteins and glycopeptides, the reaction of periodate with various α-amino acids and their derivatives has been examined in acidic, basic and unbuffered media. Oxidations of α-amino acids usually occur by the general reaction:

$$R \cdot CH(NH_2) \cdot CO_2H + O \rightarrow R \cdot CHO + NH_3 + CO_2$$

although this process is generally slow for periodate. Oxidation is facilitated by the presence of a neighbouring electron-releasing group, as shown by Nicolet & Shinn (1939), who found that, although many α-amino acids were oxidized by periodate, the reaction was slow except with serine, threonine, tryptophan, methionine and cysteine. The ease with which the β-hydroxy-α-amino acids serine, threonine and hydroxylysine are oxidized has formed the basis of a method for their quantitative estimation (Shinn & Nicolet, 1941; Van Slyke, Hiller & MacFadyen, 1941; Martin & Synge, 1941; Rees, 1946). Other α-amino acids that are oxidized include histidine, proline and hydroxyproline (Carter & Neville, 1947; Carter & Loo, 1948; Bragg & Hough, 1958; Skursky, 1959). The oxidation of amino acid residues when they are combined as in peptides and proteins has been less well studied. Oxidation with periodate has been shown to cause loss of biological activity in many proteins, including ribonuclease, immune globulins and virus (Goebel, Olitsky & Saenz, 1946), lysozyme (Maekawa & Kushibe, 1955), lactate dehydrogenase (Nygaard, 1956), glucosidase and phosphatase (Bossard, 1948).

* Present address: Department of Medicine, Bristol Royal Infirmary, Bristol, 2.

When ovalbumin, the egg-white glycoprotein, was oxidized by periodate for a short period without causing denaturation, only cysteine and tryptophan residues were appreciably destroyed, and tyrosine, histidine and cystine were largely unaffected (Maekawa & Kushibe, 1954). When ovalbumin was oxidized more extensively at pH 5·5 until it was denatured (Desnuelle, Antonin & Casal, 1947), it consumed approx. 30 mol. of periodate, and lost the cysteine and cystine residues, 1 mol. of tryptophan and 1 mol. of tyrosine, but the methionine was said to be intact. Desnuelle *et al.* (1947) calculated that the oxidation of these amino acids accounted for 20 mol. of periodate and suggested that the other 10 mol. might in part have oxidized proline. The extra periodate can, however, be accounted for by oxidation of the carbohydrate prosthetic group or histidine residues or both. The periodate oxidation of collagen (Zahn & Zürn, 1957) and procollagen (Hormann, Hannig & Fries, 1959) led to the loss of hydroxylysine, cystine, tyrosine and methionine residues. Periodate has been used to oxidize the *N*-terminal serine residue of corticotrophin (Dixon, 1962).

METHODS

Periodate oxidation. The compound to be oxidized was accurately weighed (approx. 0·25 m-mole) and transferred to a 100 ml. volumetric flask. When required, the buffer solution (25 ml. of 0·1 M-potassium tetraborate solution for pH 9·0; 25 ml. of 0·1 N-H_2SO_4 for pH 2·0) was then added. After the addition of 0·3 M-sodium metaperiodate solution (5 ml.), the mixture was quickly made up to volume with water and kept in the dark at room temperature. For oxidation at other pH values, the compound and the sodium metaperiodate solution were added separately to distilled water (approx. 40 ml.) and each was adjusted to the required pH with 0·1 N-H_2SO_4 or 0·1 N-KOH. The two solutions were

Table 1. *Periodate oxidation of glycine and derivatives*

Experimental details are given in the text. *N*-Acetylglycine and *NNN*-trimethylglycine (glycine betaine) were not oxidized by periodate under any of these conditions, or at pH 12·0.

	Consumption of periodate (mol./mol. of compound)							
	(*A*) pH 2·0		(*B*) Unbuffered				(*C*) pH 9·0	
Time (hr.)	Glycine	*N*-Methyl-glycine	Glycine	*N*-Methyl-glycine	Glycine ethyl ester	Glycyl-glycine	Glycine	*N*-Methyl-glycine
6	0·09	0·20	0·16	0·30	—	—	0·14	0·36
46	0·12	0·46	0·18	0·84	0·38	0·37	0·18	0·76
75	0·13	0·62	0·23	1·00	0·40	0·33	0·31	0·90
100	0·13	0·70	0·29	1·10	0·36	—	0·30	1·00
175	0·13	0·81	0·31	1·30	0·38	0·37	0·26	1·25
250	0·13	0·90	0·37	1·43	0·38	0·40	0·32	1·43
340	0·13	0·95	0·45	1·54	—	—	0·38	1·58
Final pH	...	...	(6·75)	(7·60)	(6·25)	(5·5)		

then mixed and made up to volume. For unbuffered oxidations, the pH of the final reaction mixture was determined with a model 23A direct-reading pH-meter (Electronic Instruments Ltd.). For each pH series, a blank was run concurrently, and 5 ml. portions were taken at intervals for determination of unused periodate by thiosulphate (Neumüller & Vasseur, 1953). In this method the sample was added to 25 ml. of 0·067 M-phosphate buffer, pH 6·9, containing potassium iodide (approx. 0·5 g.), and the liberated iodine titrated with 0·01 M-sodium thiosulphate solution. The rate of CO_2 production during the oxidation was also measured. The compound to be oxidized was accurately weighed (approx. 0·025 m-mole) and transferred to the main compartment of a Warburg flask, together with distilled water (9·5 ml.) or 0·025 N-H_2SO_4 (9·5 ml.). 0·3 M-Sodium metaperiodate solution (0·5 ml.) was added to the side arm, and after temperature equilibration the two solutions were mixed and the amount of CO_2 evolved was followed.

Amino acid derivatives. *NN*-Dimethylproline was prepared by the method of King (1941).

N-Acetylated amino acids and amino acid esters were prepared according to the method of Vogel (1959).

2-Hydroxymethylpyrrolidine was kindly provided by Professor W. D. Ollis and *O*-benzoylthreonine by Dr P. D. Bragg.

L-Amino acids were supplied by British Drug Houses Ltd.

RESULTS AND DISCUSSION

All results for the consumption of oxidant, or liberation of carbon dioxide, are given in Tables 1–9 and are quoted as mol./mol. of compound. The production of a precipitate or a coloured solution during oxidation is indicated in each Table when it occurred. In those oxidations carried out under unbuffered conditions, the pH of the final solution is also shown. All the oxidations were carried out under exactly the same conditions of temperature and concentration of reactions.

The oxidation of glycine and some of its derivatives was studied as an example of the effect of periodate on those amino acids containing no functional group other than the carboxyl and α-amino groups. The results in Table 1 show that glycine was oxidized at a low rate under acidic, alkaline and unbuffered conditions, and that mono-*N*-methylation increased the rate and extent of oxidation. The presence of a hydrogen substituent on the nitrogen atom appears to be necessary for oxidation to occur in acidic solution, since *NN*-dimethylglycine was oxidized only in alkaline solution (Table 2). This may reflect the difference in the oxidizing species in acidic and alkaline solution (Bunton & Shiner, 1960). A positively charged nitrogen atom resists attack by periodate, probably by electrostatic repulsion of a positively charged oxidant (Levitt, 1955), and it has been found (Cantley & Hough, 1963) that the rate of oxidation of 2-aminoethanol and other α-amino alcohols decreases with decreasing pH, owing to the increase in the proportion of the protonated species.

Table 2. *Effect of* pH *on the oxidation of* NN-*dimethylglycine*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of *NN*-dimethylglycine)					
	pH 2·0	pH 6·4	pH 7·0	pH 8·4	pH 9·0	pH 11·35
1	0·0	0·0	—	—	—	0·25
6	0·0	0·0	—	0·08	0·09	—
25	0·0	0·0	0·04	—	—	0·87
46	0·0	0·0	0·06	0·44	0·73	1·30
75	0·0	0·0	—	0·67	0·96	1·73
100	0·0	0·0	0·07	0·81	1·05	2·05
175	0·0	0·0	0·09	1·03	1·37	—
250	0·0	0·0	—	1·22	—	—

Table 3. *Periodate oxidation of hydroxyamino acids and derivatives*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of compound) Serine	Glycylserine	Threonine	*O*-Benzoylthreonine
(*A*) pH 2·0				
3	0·49	0·26	1·05	0·31
25	1·77	0·28	1·44	0·27
51	2·00	0·23	1·99	0·31
98	2·04	0·28	2·13	0·31
170	2·04	0·33	2·16	0·31
(*B*) Unbuffered				
3	1·90	0·29	1·99	—
25	2·02	0·26	1·99	0·30
51	2·05	0·30	2·00	0·30
98	2·01	0·24	2·00	0·30
170	2·00	0·34	2·09	0·38
Final pH ...	(5·70)	(4·95)	(5·70)	(5·85)
(*C*) pH 9·0				
3	1·68	0·65	1·79	0
25	2·27	0·92	3·24	0
51	2·30	1·07	3·22	0
98	2·31	1·23	3·15	0
170	2·36	1·33	3·23	0

For the same reason *NNN*-trimethylglycine (glycine betaine), which has a formal positive charge on the nitrogen atom, was not oxidized in either acidic or alkaline media. Similarly *N*-acetylglycine was stable to periodate, since the amino group is replaced by an electron-withdrawing group. Esterification of the carboxyl group appeared to have little effect on the rate of oxidation.

Both serine and threonine consumed 2 mol. of periodate in acidic solution, but the uptake was greater at pH 9·0, being 3 mol. for threonine (Table 3). At pH 2·0 both amino acids reacted rapidly with 1 mol. of oxidant, followed by the slower uptake of a further 1 mol., which was accompanied by the release of 1 mol. of carbon dioxide (Table 9). Under unbuffered conditions (pH 5·7) both the 2 mol. of periodate were consumed rapidly. Substitution of the amino group of serine as in glycylserine, and of the hydroxyl group of threonine as in *O*-benzoylthreonine, limited the uptake of periodate to that of the free amino group (0·3 mol./170 hr.). This suggests that both the amino and hydroxyl groups must be unsubstituted for the rapid oxidation of serine and threonine, and that these amino acids would not be attacked during the periodate oxidation of a protein unless they occurred as *N*-terminal units.

Table 4. *Periodate oxidation of sulphur-containing amino acids and related compounds*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of compound) 2-Aminoethanethiol hydrochloride (cysteamine)	Half residue of 1,1′-dithiobis-(2-aminoethane) dihydrochloride (cystamine)	Taurine	Cysteine hydrochloride	Half residue of cystine	Glutathione	Methionine	*N*-Acetylmethionine
(*A*) pH 2·0								
6	2·98	—	0·22	1·90	0·78	2·05	1·18	0·98
28	3·71	—	0·30	2·08	2·20	2·35	1·24	—
54	3·68	—	0·24	2·12	2·41	2·43	1·33	1·03
76	3·72	—	0·26	2·24	2·43	2·46	1·37	1·05
125	—	—	—	2·35	2·58	2·46	—	1·05
151	—	—	—	2·45	2·60	2·50	—	—
(*B*) Unbuffered								
6	3·11	1·42	0·22	2·03	0·50	2·10	1·23	0·88
28	4·33	2·76	0·27	2·37	1·49	2·39	1·41	1·04
54	4·40	3·75	0·25	2·50	2·25	2·57	1·56	1·07
76	4·45	3·95	0·24	2·57	3·05	2·67	1·78	1·09
125	—	—	—	2·61	3·20	2·85	—	1·15
151	4·70	4·00	—	2·68	3·25	2·89	—	—
Final pH ...	(2·70)	(2·65)	(5·30)	(2·82)	(3·30)	(3·32)	(6·1)	(3·30)
(*C*) pH 9·0								
6	3·18	1·74	0·29	2·70	1·32	2·30	1·54	1·03
28	4·50	3·65	0·37	3·42	2·43	2·37	1·57	1·03
54	5·10	4·52	0·42	3·66	2·66	3·28	1·60	1·00
76	5·35	4·90	0·45	3·74	2·78	3·38	1·64	1·10
125	—	—	—	3·86	2·85	3·42	—	1·13
151	—	—	—	3·88	2·90	3·50	—	—

Apart from the observation of Nicolet & Shinn (1939) that cysteine and methionine are oxidized by periodic acid, probably through the sulphur atom, little further work has been done on the sulphur-containing amino acids. However, the periodate oxidation of sulphur-containing carbohydrates has been investigated (Hough & Taha, 1956, 1957*a*, *b*), and it was found that an ethylthio group consumed 1 mol. In acidic solution, there was a rapid uptake of 2 mol. of periodate by free thiol groups as in cysteine and glutathione, but an uptake of 3 mol. by a similar group in 2-aminoethanethiol (cysteamine) (Table 4). These compounds were oxidized further, especially in alkaline solution. Cystamine and cystine gave similar uptakes to, but were oxidized at a slower rate than, 2-aminoethanethiol (cysteamine) and cysteine respectively. The methylthio groups of methionine and *N*-acetylmethionine, on the other hand, reacted with only 1 mol. of periodate, the slightly greater uptake by methionine probably being due to slow oxidation of the amino group (this is negligible in the *N*-acetyl compound). Methionine, cysteine and cystine released very little carbon dioxide during their oxidations, in accordance with sulphur being the primary centre of attack. Since oxidation occurs at the sulphur atom and is not dependent on a free amino or carboxyl group, cysteine, cystine and methionine would be oxidized wherever they occurred in a peptide chain, and this was confirmed by the similar uptake of periodate by glutathione.

Bragg & Hough (1958) found that at pH 2·2 proline consumed 1 mol. of periodate, releasing 1 mol. of carbon dioxide, and that a small amount of Δ^1-pyrroline could be isolated from the solution. The yield of Δ^1-pyrroline can be made virtually quantitative by the addition of mercuric chloride or 2-aminobenzaldehyde (Skursky, 1959). At a higher pH proline reacted with 2 mol. of periodate, and pyrrolid-2-one was isolated from the reaction mixture. Periodate uptake and carbon dioxide production have now been confirmed, although at pH 2·0 proline was found to consume more than 1 mol. of periodate (Table 5). 2-Hydroxymethylpyrrolidine gave results similar to those for proline, but when the nitrogen atom was substituted, as in

Table 5. *Periodate oxidation of pyrrolidine-containing amino acids and related compounds*

Experimental details are given in the text. Stachydrine (proline betaine) did not consume periodate under any of these conditions.

Time (hr.)	Consumption of periodate (mol./mol. of compound)				
	Proline	Hydroxyproline	Glycylproline	2-Hydroxymethyl-pyrrolidine	Pyrrolidine
(*A*) pH 2·0					
2	0·31	0·55	0·26	0·32	0·04 (5 hr.)
24½	1·32	2·15	0·32	1·35	0·08 (29 hr.)
50½	1·44	2·35	0·26	—	0·10 (53 hr.)
74	1·46	2·47	0·26	1·44	—
98	1·50	2·42	—	—	0·09 (101 hr.)
209	1·58	2·65	—	1·50	—
Ppt.	(−)	(+)	(−)	(−)	(−)
Soln. colour ...	(−)	(Yellow)	(−)	(−)	(−)
(*B*) Unbuffered					
2	0·70	1·50	0·22	1·85	0·07 (5 hr.)
24½	1·94	3·53	0·20	2·05	0·12 (29 hr.)
50½	1·95	3·62	0·33	—	0·12 (53 hr.)
74	2·08	3·67	0·40	2·05	—
98	—	—	—	—	0·12 (101 hr.)
209	2·13	3·83	—	2·05	—
Final pH ...	(5·54)	(3·73)	(6·33)	(5·05)	(7·5)
Soln. colour ...	(−)	(Yellow)	(−)	(−)	(−)
(*C*) pH 9·0					
2	0·18	0·93	0·40	1·87	0·12 (5 hr.)
24½	0·82	2·67	0·52	2·22	0·22 (29 hr.)
50½	1·23	3·32	0·58	—	0·16 (53 hr.)
74	1·55	3·45	0·64	2·22	—
98	1·85	3·59	—	—	0·16 (101 hr.)
209	2·31	3·82	—	2·24	—

glycylproline, the uptake of periodate was drastically reduced. The parent ring structure pyrrolidine showed only a slight uptake, indicating that in the oxidation of proline and hydroxymethylpyrrolidine the carboxyl and hydroxymethyl groups have a similar action as electron-releasing groups for oxidation at the secondary amino group. Hydroxyproline was more extensively oxidized, consuming 3 mol. of periodate and releasing 1 mol. of carbon dioxide at pH 2·0. In more alkaline solutions the consumption rises to 4 mol. Proline betaine (stachydrine), which carries a formal positive charge on the nitrogen atom, was not oxidized under acidic or basic conditions.

Nicolet & Shinn (1939) first noted that tryptophan reacted rapidly with more than 1 mol. of periodate to form an insoluble product. Tryptophan yielded a scanty black precipitate after periodate oxidation at pH 2·0, although not at higher pH values. The consumption of 4 mol. of periodate at pH 2·0 (Table 6) was accompanied by the release of 1 mol. of carbon dioxide (Table 9). This uptake was diminished by acylation of the α-amino group as in *N*-acetyltryptophan and glycyltryptophan, indicating that both the indole nucleus and the alanine side chain were involved in the overall oxidation of this amino acid. Indole had a similar uptake to these tryptophan derivatives, supporting the idea that initially there was a rapid reaction with the indole nucleus followed by a slower reaction involving the alanine side chain. Though *N*-acetylindole was not expected to consume periodate, investigation revealed a slight uptake, but this was probably due to the oxidation of the unsaturated 5-membered ring, since indene showed similar behaviour.

Whereas tyrosine was extensively oxidized by periodate, phenylalanine was not, suggesting that the phenolic group is important in this reaction (Table 7). Phenol itself was only slowly attacked by periodate, but substitution with a methyl group in the *para*-position (*p*-cresol) increased the rate and

Table 6. *Periodate oxidation of tryptophan and related compounds*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of compound) Tryptophan	*N*-Acetyltryptophan	Glycyltryptophan	Time (hr.)	Consumption of periodate (mol./mol. of compound) Indole	*N*-Acetylindole	Indene
(*A*) pH 2·0							
2	2·54	0·17	0·69	5	0·94	0	0
22½	3·12	1·50	2·35	28	1·28	0	0·09
47½	3·35	2·06	2·47	52¾	1·53	0	0·12
94½	3·66	2·33	3·21	77	1·68	0·11	0·28
118	3·88	2·37	3·33	100	1·76	0·20	0·41
				172	2·08	—	0·87
Ppt. ...	(+)	(−)	(−)		(+)	(−)	(−)
Soln. colour	(Brown)	(Orange)	(Yellow)		(Brown)	(Orange)	(−)
(*B*) Unbuffered							
2	2·60	0·14	1·01	5	1·37	0·46	0
22½	3·74	1·70	2·07	28	1·98	0·67	0·13
47½	4·03	2·28	2·30	52¾	2·12	0·69	0·35
94½	4·59	2·59	2·46	77	2·26	0·76	0·55
118	4·74	2·76	2·58	100	2·36	0·86	0·87
				172	2·66	—	1·70
Final pH ...	(4·23-	(3·44)	(4·04)		(3·55)	(7·0)	(3·55)
Ppt. ...	(−)	(−)	(−)		(+)	(−)	(−)
Soln. colour	(Yellow)	(Red)	(Yellow)		(Brown)	(Yellow)	(Yellow)
(*C*) pH 9·0							
2	2·61	0·10	0·42	5	1·03	0·64	0·14
22½	2·85	0·91	1·19	28	1·23	0·83	0·59
47½	3·30	1·55	1·70	52¾	1·39	0·90	0·98
94½	3·90	2·14	3·05	77	1·62	0·91	1·40
118	3·97	2·42	3·00	100	1·70	0·90	1·89
				172	1·96	—	2·67
Soln. colour	(Orange)	(Yellow)	(Yellow)		(Brown)	(Yellow)	(Yellow)

Table 7. *Periodate oxidation of tyrosine and related compounds*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of compound)					
	Phenol	*p*-Cresol	4-Methoxy-toluene	Phenyl-alanine	Tyrosine	Glycyl-tyrosine
(*A*) pH 2·0						
4	0·09	2·10	—	—	0·19	0·80
28	0·45	2·74	0·18	0·08	0·96	2·31
52	0·45	3·29	0·18	0·08	1·48	3·54
76	0·61	3·18	0·27	0·12	1·89	4·07
100	—	3·22	—	0·17	—	4·40
164	1·18	3·00	0·21	0·22	2·96	4·92
268	—	—	—	—	3·52	5·66
Ppt. ...	(+)	(−)	(−)	(−)	(+)	(−)
Soln. colour	(Yellow)	(Orange)	(−)	(−)	(Orange)	(Yellow)
(*B*) Unbuffered						
4	0·04	0·97	0·05	0·34	0·23	0·80
28	0·47	2·47	0·10	0·34	0·37	1·02
52	0·58	3·13	0·12	0·43	0·85	1·45
76	0·62	3·45	0·14	0·40	1·16	1·81
100	0·63	3·61	—	0·52	1·58	2·47
164	0·68	3·83	0·17	0·74	2·61	3·37
268	—	—	—	—	3·34	4·29
Final pH ...	(4·40)	(3·20)	(4·35)	(5·68)	(3·47)	(3·37)
Ppt. ...	(+)	(−)	(−)	(−)	(−)	(−)
Soln. colour	(−)	(Orange)	(−)	(−)	(Orange)	(Yellow)
(*C*) pH 9·0						
4	0·41	0·91	0·13	—	0·68	1·27
28	1·10	—	0·09	0·28	1·53	1·39
52	0·98	1·20	0·05	0·48	2·30	2·05
76	1·20	1·17	0·09	0·44	2·65	2·19
100	1·29	1·09	—	0·62	3·04	2·46
164	1·41	1·14	0·09	0·86	3·69	2·91
268	—	—	—	—	4·03	3·30
Ppt. ...	(+)	(+)	(−)	(−)	(−)	(−)
Soln. colour	(Brown)	(−)	(−)	(−)	(Orange)	(Yellow)

extent of the oxidation. When the hydroxyl group was methylated, as in 4-methoxytoluene, the uptake of periodate was drastically reduced. Substitution of the amino group of tyrosine by glycine (glycyltyrosine) appeared to increase the consumption of periodate in acidic solution.

The dicarboxylic amino acids, such as glutamic acid, showed a slow uptake of periodate owing to the oxidation of the α-amino group, but lysine as representative of the dibasic amino acids had a more rapid consumption owing to the extra amino group present (Table 8). The guanidino group in arginine and creatine was not attacked by periodate, presumably because of the positive charge on the nitrogen atom, which is stabilized by resonance. Histidine was rapidly oxidized by periodate, consuming 1 mol. at pH 2·0 but more at higher pH values. The uptake by histidine was greater than that of imidazole and indicates that, as with tryptophan, oxidation involves both the heterocycle and the alanine side chain, the former being attacked more rapidly.

The above studies indicate that the following amino acids would be oxidized by periodate wherever they occurred in the peptide chain: cysteine, cystine, methionine, tryptophan, tyrosine and histidine; serine and threonine would only be attacked as *N*-terminal residues. Since so many amino acids are attacked, it is not surprising that periodate denatures proteins and inactivates enzymes. In addition, periodate oxidation studies applied to glycoproteins and glycopeptides must be interpreted with caution if these amino acids are present. Provided that the content of oxidizable amino acids is small and appropriate controls are determined, the consumption of periodate and acid

Table 8. *Periodate oxidation of miscellaneous amino acids and compounds*

Experimental details are given in the text.

Time (hr.)	Consumption of periodate (mol./mol. of compound)					
	Glutamic acid	Lysine hydrochloride	Imidazole	Histidine hydrochloride	Arginine hydrochloride	Creatine
(*A*) pH 2·0						
4	—	0·22	0·05	0·22	0·19	0·0
28	0·09	0·24	0·07	0·85	—	0·0
53	—	0·29	0·03	—	—	0·0
78	0·11	—	0·01	—	0·22	0·0
101	—	0·43	0·0	—	—	0·0
174	0·19	—	—	1·19	0·42	—
(*B*) Unbuffered						
4	0·02	—	0·45	0·32	0·18	0·0
28	0·09	0·44	0·55	0·67	0·28	0·0
53	—	0·61	0·69	—	—	0·0
78	0·30	—	0·78	1·45	0·54	0·0
101	—	1·08	0·83	—	—	0·0
174	0·46	—	—	2·38	0·75	—
Final pH ...	(4·40)	(5·20)	(7·05)	(3·85)	(6·75)	(5·45)
(*C*) pH 9·0						
4	0·15	0·35	1·13	2·86	0·40	0·0
28	—	0·37	1·44	3·33	0·45	0·0
53	—	0·41	1·21	—	—	0·0
78	0·16	—	1·26	3·45	0·68	0·0
101	—	0·63	1·31	—	—	0·0
174	0·24	—	—	3·62	0·71	—

Table 9. *Evolution of carbon dioxide during periodate oxidation of amino acids at* pH 2·0

Experimental details are given in the text.

Time (hr.)	CO_2 production (mol./mol. of compound)									
	Proline	Hydroxy-proline	Cysteine	Half residue of cystine	Methionine	Histidine	Serine	Threonine	Tyrosine	Tryptophan
0·5	0·13	0·13	0	0·0	0	0·03	0·02	0·03	0·0	0·01
1·2	0·30	0·29	0	0·01	0	—	0·12	0·14	—	0·10
1·5	0·37	0·38	0·01	0·02	0·01	0·03	0·19	0·21	0·01	0·16
2·5	0·50	0·51	0·02	0·03	0·01	0·04	0·34	0·38	—	0·30
3·0	0·55	0·56	—	0·04	—	—	0·39	0·45	0·01	0·33
4·0	0·64	0·66	0·02	0·05	0·01	0·04	0·50	0·57	—	0·40
5·5	0·73	0·75	0·03	0·06	—	0·05	0·62	0·69	0·02	0·46
8·5	0·82	0·85	0·04	0·08	0·02	0·06	0·74	0·80	0·04	0·49
21·5	0·89	0·94	0·08	0·10	0·04	0·12	0·80	0·86	0·16	0·55
25·0	—	—	—	0·10	0·05	0·14	—	0·87	0·18	0·56
30·0	—	—	0·10	0·11	0·06	0·16	0·81	—	0·24	0·59
45·0	—	—	0·11	0·12	0·07	0·22	—	—	0·33	0·60
48·0	—	—	0·12	0·12	0·07	0·23	0·82	0·88	0·36	0·62
55·0	—	—	0·15	0·14	0·09	0·27	—	—	0·42	0·65
70·0	0·93	0·98	0·17	0·15	0·11	0·31	0·85	0·89	0·55	0·69

liberation by the carbohydrate moiety in a glycopeptide may be calculated by difference. Clearly the most reliable evidence is obtained by analysis of those monosaccharide units that remain after the rapid phase of oxidation is complete.

The Medical Research Council Scholarship awarded to J.R.C. is gratefully acknowledged.

REFERENCES

Bossard, M. (1948). *Année biol.* **52**, 202.
Bragg, P. D. & Hough, L. (1958). *J. chem. Soc.* p. 4050.
Bunton, C. A. & Shiner, V. J. (1960). *J. chem. Soc.* p. 1593.
Cantley, M. & Hough, L. (1963). *J. chem. Soc.* p. 2711.
Carter, H. E. & Loo, Y. H. (1948). *J. biol. Chem.* **174**, 723.
Carter, H. E. & Neville, H. E. (1947). *J. biol. Chem.* **170**, 301.
Desnuelle, P., Antonin, S. & Casal, A. (1947). *Bull. Soc. Chim. biol., Paris*, **29**, 694.
Dixon, H. B. F. (1962). *Biochem. J.* **83**, 91.
Goebel, W. F., Olitsky, P. K. & Saenz, A. C. (1946). *J. exp. Med.* **87**, 445.
Hormann, H., Hannig, K. & Fries, G. (1959). *Hoppe-Seyl. Z.* **315**, 109.
Hough, L. & Taha, M. I. (1956). *J. chem. Soc.* p. 2042.
Hough, L. & Taha, M. I. (1957*a*). *J. chem. Soc.* p. 3311.
Hough, L. & Taha, M. I. (1957*b*). *J. chem. Soc.* p. 3994.
King, H. (1941). *J. chem. Soc.* p. 337.
Levitt, L. S. (1955). *J. org. Chem.* **20**, 1297.
Maekawa, K. & Kushibe, M. (1954). *Bull. chem. Soc. Japan*, **27**, 277.
Maekawa, K. & Kushibe, M. (1955). *Bull. agric. chem. Soc. Japan*, **19**, 28.
Martin, A. J. P. & Synge, R. L. M. (1941). *Biochem. J.* **35**, 294.
Neumüller, G. & Vasseur, E. (1953). *Ark. Kemi*, **5**, 235.
Nicolet, B. H. & Shinn, L. A. (1939). *J. Amer. chem. Soc.* **61**, 1615.
Nygaard, A. P. (1956). *Acta chem. scand.* **10**, 397.
Rees, R. W. (1946). *Biochem. J.* **40**, 632.
Shinn, L. A. & Nicolet, B. H. (1941). *J. biol. Chem.* **138**, 91.
Skursky, L. (1959). *Z. Naturf.* **14**b, 473.
Van Slyke, D. D., Hiller, A. & MacFadyen, D. A. (1941). *J. biol. Chem.* **141**, 681.
Vogel, A. I. (1959). *A Textbook of Practical Organic Chemistry*, 3rd ed. pp. 424–427. London: Longmans, Green and Co. Ltd.
Zahn, H. & Zürn, L. (1957). *Z. Naturf.* **12**b, 788.